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SUGGESTIONS FOR CHANGES IN THE SCHEDULES FOR MAKING BROTH, GELATIN, AND AGAR, RECOMMENDED IN THE LAST REPORT OF THE COMMITTEE ON STANDARD METHODS OF WATER ANALYSIS.¹

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Suggestion 1.—In preparing broth, gelatin, and agar, transfer the direction to "adjust to the required reaction," from its present position to precede immediately the direction for heating over the water bath for half an hour.

The reason for this change is that, in order to secure uniformity in successive lots of media, it is well that all heating should be done at a uniform reaction. If the recommendations as they stand now are followed, the half-hour's heating on the water bath is given before the adjustment to a fixed reaction, i. e., at the casual reaction which the meat infusion may happen to have, further modified somewhat by the addition of peptone in all cases, and, in the case of gelatin, very much modified by the presence of gelatin. If, however, the change here suggested be adopted, the reaction at which all heating is done will be the same throughout for all media which are to have the same final reaction. It will be remembered that the earlier recommendations of this Committee called for neutralization before heating, and this provided that the reaction at which all heating was done should be the same throughout for all media, without regard to the final reaction. My suggestion, if adopted, will return as closely as may be to uniformity under the modified conditions as regards the final reaction now generally adopted, i. e., the adjustment to a final reaction without previous neutralization.

Suggestion 2.—In preparing broth, gelatin, and agar, make the period of boiling over the free flame five minutes instead of two

¹ Jour. Infect. Dis., 1905, Supplm. No. 1, p. 1.

minutes as in the present recommendation. This also is a return to the recommendations formerly made.

The reason for this change is, that it seems to be a fairly common experience that two minutes' boiling is hardly sufficient to precipitate thoroughly the albumens present at the reaction at which the boiling is done. Five minutes' boiling, on the other hand, is usually sufficient for precipitation, and obviates subsequent precipitation during sterilization.

Suggestion 3.—Since, in the adjustment of agar media by titration, some little difficulty is encountered at times, if it be done after the addition of the 3 per cent agar, and especially if the titration process be prolonged, because of the agar becoming cold, it is at times convenient to adjust the reaction of the meat infusion plus peptone before adding the agar. If this be done, the reaction to which the meat infusion is adjusted should be double that which is desired as the final reaction. The subsequent addition of the agar in 3 per cent strength, as recommended, brings the reaction, as well as the percentage of peptone and of meat infusion constituents, to the proper point. Since it is difficult to see that the adjustment of the agar reaction before or after the addition of agar can make any difference in the composition of the medium, it would seem that an alternative method might here be provided without infringing on the principle of securing strict uniformity. The 3 per cent agar being neutral, exactly the same amount of alkali is necessary to secure the same final reaction from either the meat infusion plus peptone, (double strength) brought to double the final reaction, and then diluted one-half by the 3 per cent agar or from the whole agar medium (final strength) brought directly to the final reaction.

Summed up these suggestions would be as follows:

- 1. In the present form of recommendations for gelatin and agar, p. 108 of Supplement No. 1 of the *Journal of Infectious Diseases*, strike out steps 11 and 12; and insert them again, renumbered 9 and 10, respectively; strike out 9 and 10 as they now are, and reinsert them, renumbering them 11 and 12, respectively.
- 2. In step 13, strike out "two minutes" and substitute "five minutes."

3. Insert a footnote, referring to steps 8, 9, 10, as renumbered in accordance with the above suggestions, to read:

If preferred, meat infusion and peptone of double strength, intended for the preparation of agar media, may be adjusted to twice the final reaction desired, before the addition of the 3 per cent agar. The addition of the agar should then be made between steps 10 and 11 (as renumbered).